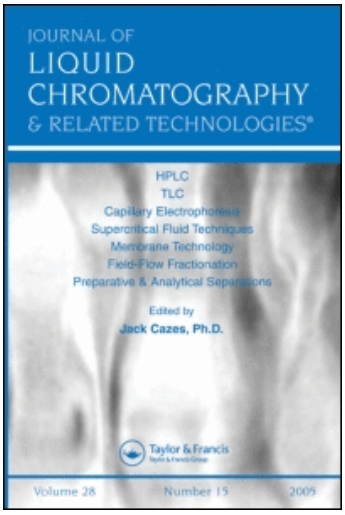

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

High-Performance Liquid Ion-Exchange Chromatography

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To cite this Article Schmuckler, Gabriella(1987) 'High-Performance Liquid Ion-Exchange Chromatography', Journal of Liquid Chromatography & Related Technologies, 10: 8, 1887 — 1901
To link to this Article: DOI: 10.1080/01483918708066804
URL: http://dx.doi.org/10.1080/01483918708066804

JOURNAL OF LIQUID CHROMATOGRAPHY, 10(8&9), 1887-1901 (1987)

HIGH-PERFORMANCE LIQUID ION-EXCHANGE CHROMATOGRAPHY

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ABSTRACT

The separation and determination of amino acids, sugars, organic acids, anions, cations, and metal complexes by this technique are reviewed, and the developments leading to its advance in recent years are discussed.

Introduction

One of the early publications stressing the promising potential of ion-exchange materials as chromatographic supports was that by Applezweig (1) in 1948. Since then this chromatographic application has made great strides, and to-day a wide variety of substances - organic and inorganic molecules such as amino acids, sugars, organic acids, cations, anions, and metal complexes - can be efficiently separated and accurately determined by this method. Not, however, until the development of very small rigid spherical ion-exchange beads (5-15 μ diameter) suitable for high-pressure operation, could this method be applied with a high degree of selectivity and sensitivity.

The microparticles referred to are divided into two groups:

1) Totally porous resinous particles usable in a wide range of pH values, including alkaline solutions;

2) Bonded silica beads, the ion-exchange groups being permanently bonded to small spherical silica-gel particles. These ion exchangers can only be used in acidic solutions; but their rigidity and fast surface interaction makes them very efficient separators with a very high number of theoretical plates in a chromatographic column.

The most widely used functional groups in these particles are either strong-base amines of the $-NR^{+}_{3}$ type (for anion separation) or strong-acid sulfonate groups of the kind, $-RSO_3^-$ (for cation exchange).

The separation mechanism is not necessarily purely electrostatic ion exchange. Additional factors, such as hydrophobic interactions and specific adsorption processes, may also play a vital role in effecting separations.

The eluants employed in Ion-Exchange Liquid Chromatography are usually dilute aqueous solutions of salts, acids, or bases; but in some cases the addition of an organic modifier greatly speeds up the migration and improves the resolution of the substances being eluted.

This paper is a review of Ion Exchange Chromatography as an analytical tool for both organic and inorganic applications.

Ion-Exchange Separation of Saccharides

Samuelson (2) was the first to show that oligosaccharides can be well resolved on a strong-base anion-exchange column in which the counter-ion is a hydrophilic anion, such as sulfate. The eluant is 75% ethanol in water, and a refractive index detector is used. Fig. 1 shows a chromatogram resulting from this system.

This separation is based on normal phase partition of the polar saccharide solute molecules between the water-rich ion-exchange column and the less polar alcoholic eluant. A similar separation can be achieved with a lithium-loaded cation exchanger, which is as hydrophilic as the sulfate-loaded anion exchanger. Shorter retention times and better resolutions can be obtained with a calcium-loaded cation-exchange resin (4). Water is the eluant, and the working temperature is 90°C.

The development of a new, extremely sensitive pulsed electrochemical detector by D. Johnson (12) has led to the production of well resolved

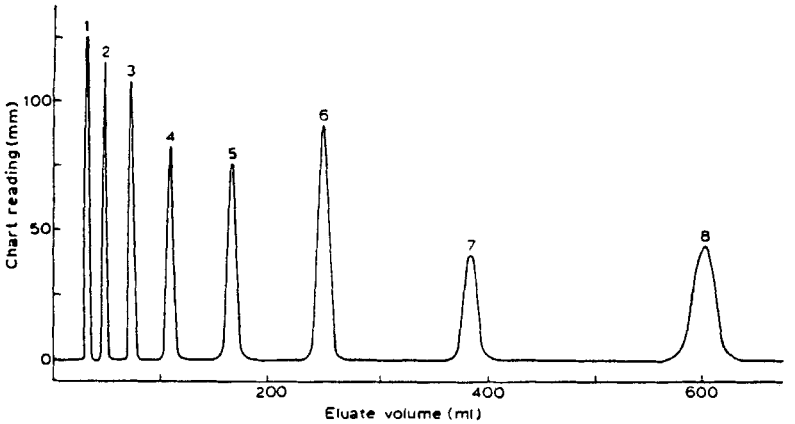

FIG. 1. Partition chromatography of xylan oligosaccharides in 75% ethanol at 75°C. Resin bed: 4 x 600 mm, Technicon T5C, SO_4^{2-}, 14 to 17 µm. Nominal linear flow: 2.8 cm/min. 1, D-xylose (5 µg); 2, di- (5 µg); 3, tri- (6.5 µg); 4, tetra- (9 µg); 5, penta- (12 µg); 6, hexa- (25 µg); 7, hepta- (18 µg); and 8, octa-saccharide (25 µg). (Ref. 2).

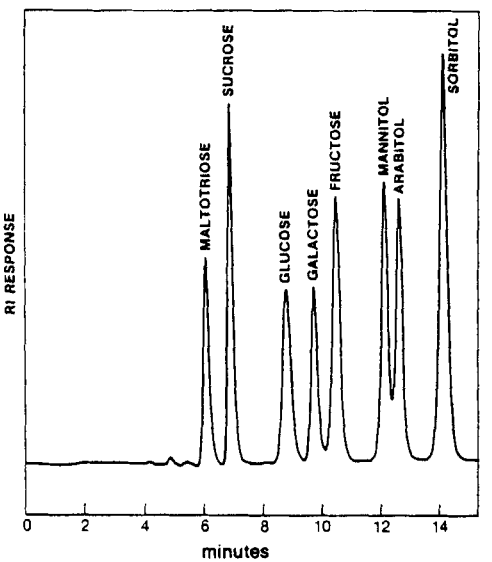

FIG. 2. Monosaccharide determination with calcium column. Column, dimensions: 0.65x30cm; eluant: filtered and degassed water; flow rate: 0.5 ml/min; temp: 90°C; detection: refractive index (Ref. 4).

chromatograms of dilute aqueous mono and disaccharide solutions. A relevant chromatogram is presented in Fig. 3.

This chromatographic separation is based on the conversion of sugars into anions by eluting with aqueous NaOH and separating the sugars on an anion-exchange column. The carbohydrates are detected amperometrically by applying a three-step potential sequence to a gold working electrode. The detection process consists of three parts:

1) Adsorption of the organic molecules and anodic oxidation of the adsorbed hydrogen:

$$R - \underset{\underset{H}{|}}{\overset{H}{|}}{C} - OH \longrightarrow (R - \underset{\bullet}{\overset{H}{|}}{C} - OH)_{ads.} + (H^{\bullet})_{ads.}$$

$$(H^{\bullet})_{ads.} \longrightarrow H^{+} + e$$

2) Oxidation of adsorbed organic molecules, which had been electrocatalyzed by Au(.OH) formed during the first step:

$$(R - \underset{\bullet}{\overset{H}{|}}{C} - OH)_{ads.} + Au(.OH) \longrightarrow Au + RCOOH + 2H^{+} + 2e$$

3) Cathodic reduction of the metal oxides covering the electrode surface. Fig. 4 illustrates this process, which is completed within 360 msecs.

The detector referred to above is entitled "Pulsed Amperometric Detector" (PAD). It performs very well under the right conditions and is sensitive to low concentrations (p.p.b.) of carbohydrates and any other organic molecules that can undergo anodic oxidation.

Among the separation methods used for saccharides the one involving a borate complex (13) should also be mentioned. It is felt, however, that the newly developed electrochemical (PAD) detector ought to prove to be more advantageous, because it is based on straightforward anion exchange, which is both rapid and accurate.

Separation of Amino Acids

High-performance liquid chromatography of amino acids was one of the major developments in ion-exchange separation, and a number of instruments,

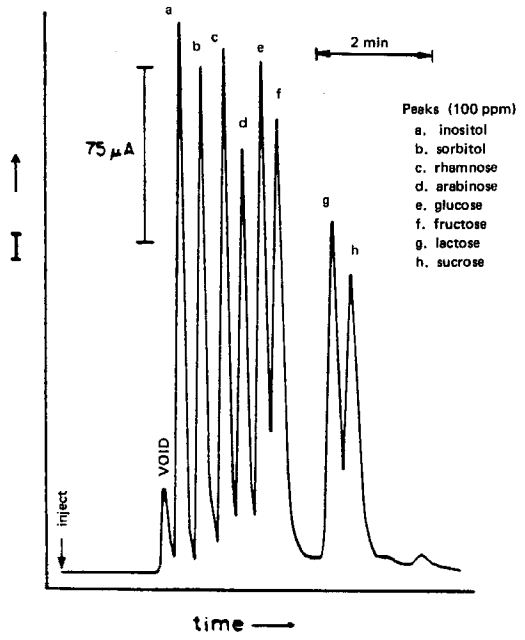

FIG. 3. Separation of oligosaccharides on an anion exchange column. Eluant: 0.15M NaOH at 36°C. (Ref. 12).

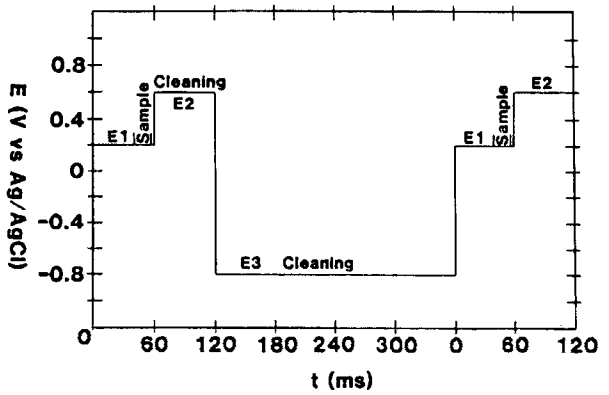

FIG. 4. Triple potential program applied to the gold working electrode for the detection of carbohydrates. Oxidation current is sampled from 40 to 56.7 ms after the beginning of E1. (Ref. 5).

specially devoted to the purpose, have been developed. Moore and Stein (6), in 1951, elaborated the first amino acid fractionation based on the differences in the pK_a values of the various amino acids. The separation is effected on a strong-acid (sulfonic) cation exchanger; and buffer solutions, the pH of which is gradually changed serve as eluants (gradient elution). In 1951 no rigid ion-exchange microparticulates were available, so that the separation was not as efficient as it is to-day. In Fig. 5(7) a typical amino-acid chromatogram, based on Moore and Stein's principles, is presented. Gradient elution is carried out with citrate and borate buffers, their pH being changed stepwise from 3 to 9.6. A photometric detector at 546 nm is used with a ninhydrin post-column reaction.

This separation initially took approximately 85 minutes; but more recently columns of greater efficiency have been devised, with which the same chromatograms can be obtained in 20 minutes (4). This time-saving is of practical importance, because in gradient elution additional time is required for the re-equilibration of the column to its original pH.

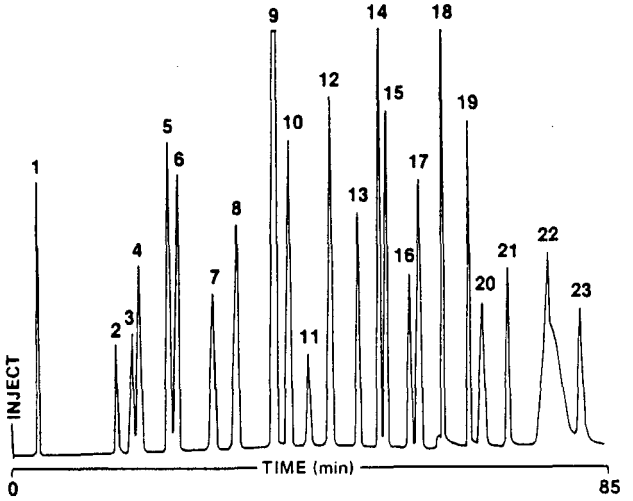

Fig. 5. Continuous gradient elution of amino acids by ion-exchange chromatography using a nonhalide buffer system. (Ref. 7).

A rival, widely used analytical method for amino acids is reversed--phase chromatographic separation with gradient elution using phosphate buffers. These separations, too, are comparatively slow, also due to the need for gradient elution. Efforts are therefore now being made to achieve faster separations of proteins and their hydrolysates by reversed-phase chromatography but eluting with micellar solutions (8). These solutions are efficient, and re-equilibration of the reversed-phase column after gradient elution does not take up much time.

The PAD electrochemical detector already mentioned, which was originally developed for the analysis of saccharides, is also well suited to the separation of amino acids (9). These are converted into anions by elution with 0.25 M NaOH and are then separated on an anion-exchange column. Fig. 6 shows a chromatogram of several amino acids, obtained with a working platinum electrode and a three-step potential wave form, which is completed within 750 msec.

Separation of Organic Acids

A wide variety of the organic acids found in food products and in biological fluids have been resolved and quantitated by two ion exchange methods. The one most commonly used is based on an exclusion process, in which the acids are separated on a sulfonic cation exchange resin in the hydrogen form. Elution with a dilute mineral acid and UV detection complete the chromatographic system. A representative example of this method is the work of Turkelson and Richards (10), who separated eight acids of the citric acid cycle, as shown in Fig. 7.

This separation was carried out on 30-35 μm particles. With the development of smaller and more rigid particles, more efficient separations have been achieved - see fig. 8, illustrating the separation of 11 acids within 13 minutes.

The other approach to organic acid separation is to convert them into anions, fractionate them on a pellicular anion exchange column, and elute them with an alkaline solution. A suppressor column is required, and the detector is of the conductivity type. The advantage of this system (fig.9) is that simple ions, such as Cl^- and $SO_4^{=}$, can be detected together with the acids.

As regards the mechanistic aspect of this separation, mixtures of low molecular weight, saturated and unsaturated acids, and hydroxy acids, can

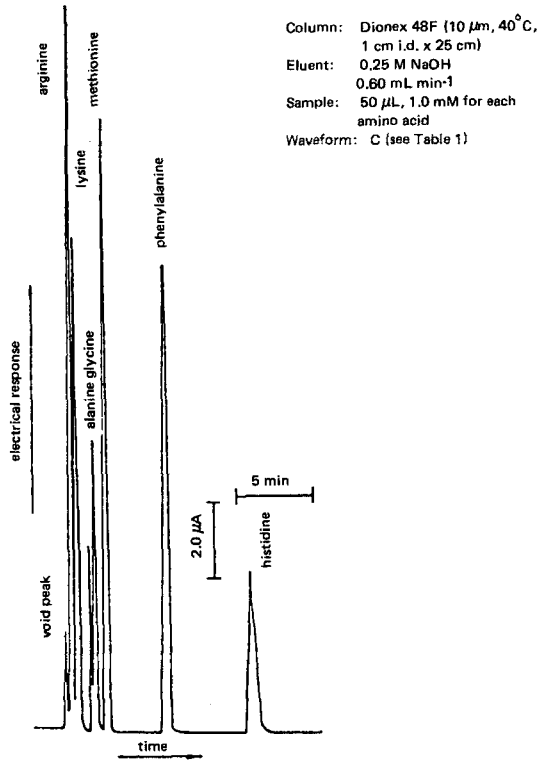

Fig. 6. Chromatogram of Selected Amino Acids Using Triple-Step Pulsed-Potential Amperometric Detection (Ref. 9).

be determined by the methods described. The main factor influencing retention times are: Molecular dimensions, acidity, and the specific adsorption of the organic acid molecules on the organic matrix of the ion exchanger. Examination of a number of chromatograms has indicated that there is, rather unfortunately, no clear-cut correlation between these factors and retention times.

Separation of Metal Ions

Metal ions can be separated on ion exchangers in either of two ways:

a) Separation as cations on rigid, low-capacity ion exchangers, and elution with acids or complexing agents;

b) Separation of anionic metal complexes on anion exchangers, and elution with ion-pairing reagents.

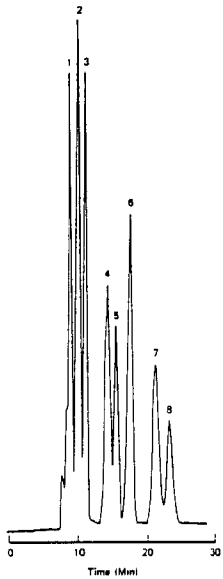

Fig. 7. Separation of citric acid cycle acids (Ref. 10).

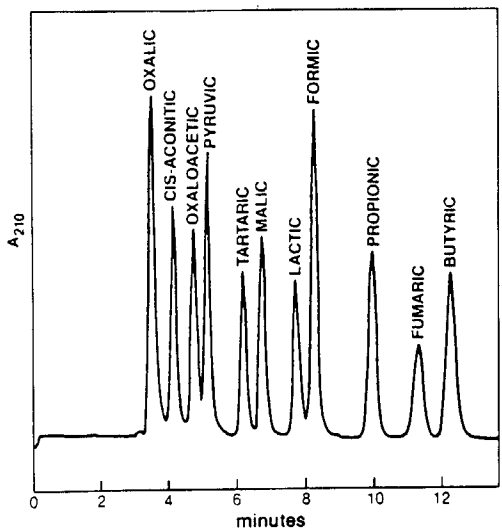

Fig. 8. Isocratic separation of organic acids. (Ref. 4).

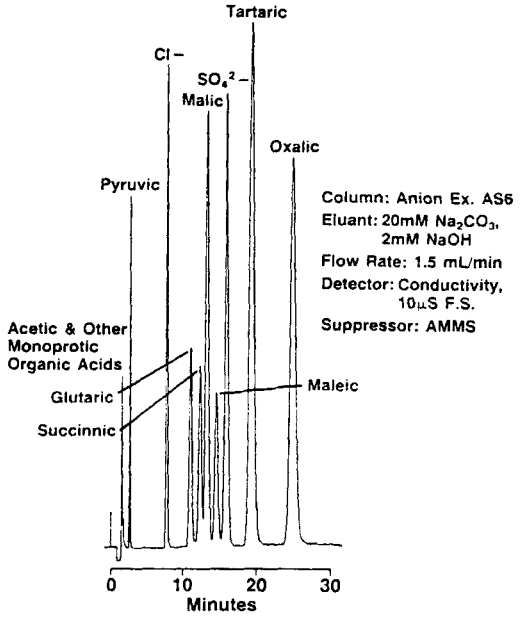

Fig. 9. Determination of diprotic organic acids by anion exchange. (Ref. 11).

For analytical purposes these separations are best applied by the technique called "Ion Chromatography" (IC), i.e. the analysis of dissolved ions by ion exchange chromatography. This is a successful combination of a separating ion-exchange column, an aqueous eluant containing acids, bases, or salts, and a sensitive conductometric detector. Small et al. (14) were the first to recognize the great potential of this technique and its application to inorganic analysis. Representative chromatograms of alkaline and alkaline earth metals are shown in figs. 10a and 10b.

The separator columns for producing these chromatograms consisted of a specially designed low-capacity cation exchanger. The eluant used for the purpose was dilute HCl, which has a high background conductivity, and a suppressor column was needed to suppress the resulting high signal. For the divalent alkaline earth metal ions (fig. 10b) a stronger eluant, such as ethylene diamine, must be used in order to overcome the electrostatic interactions in the column. Transition metal ions can be efficiently chromatographed on strong-acid cation exchangers, from which they are eluted with mixtures of ethylene diamine and hydroxy acids (15, 16).

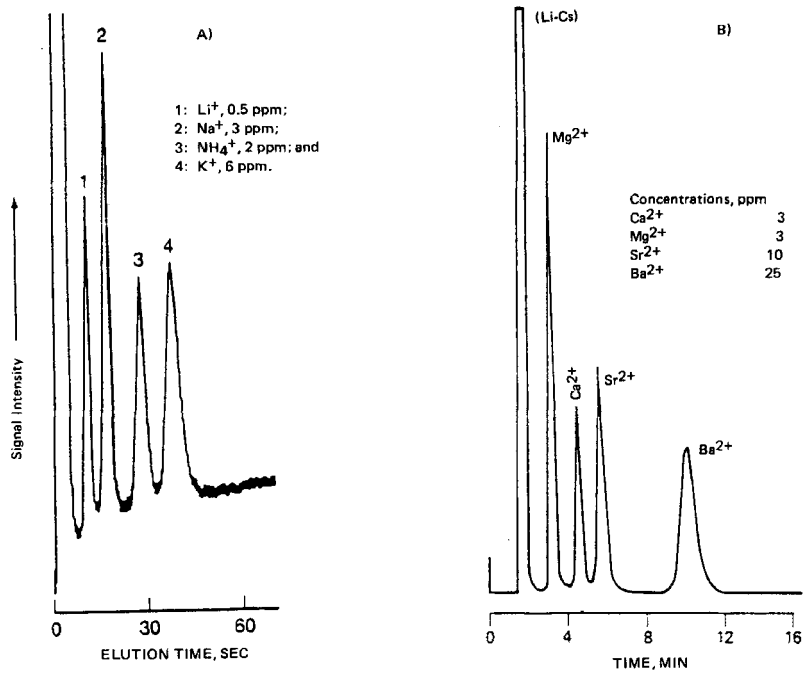

Fig. 10. Chromatographic separation of a) monovalent cations, b) divalent cations (Ref. 20).

A representative example of anionic metal complexes are the cyanide complexes of transition metals. These have been efficiently chromatographed by C. Pohlandt (17), the results being shown in fig. 11.

A major advance in metal ion separation is due to Elchuk and Cassidy (18), who succeeded in interseparating the lanthanides. They used bonded-phase sulfonic cation exchangers to separate 14 lanthanides. The eluant used was hydroxy-isobutyric acid, which forms anionic complexes with these ions. With the aid of gradient elution they generated well resolved chromatograms, one of which is shown in fig. 12.

The order of elution is dictated by the lanthanide contraction effect. Lu, which has the highest atomic number of all the lanthanides, reacts most strongly with the eluant, producing the stablest complex ion; while La, the element with the lowest atomic number, elutes last.

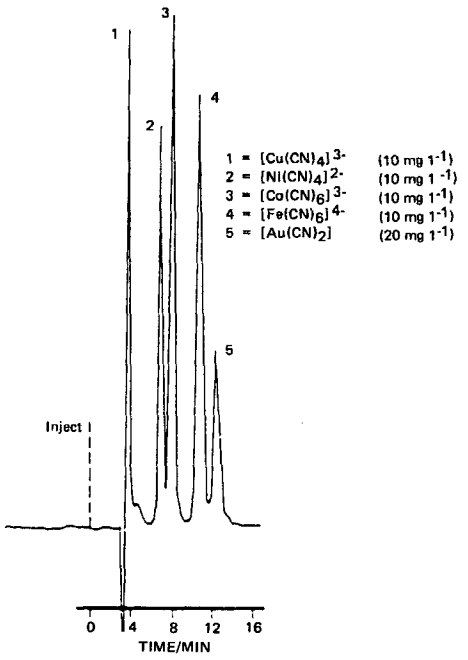

Fig. 11. The separation of five individual cyanide complexes (Ref. 17).

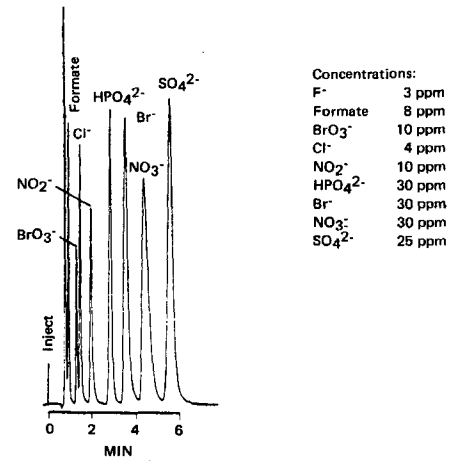

Fig. 12. An Ion-Chromatogram of nine common anions (Ref. 19).

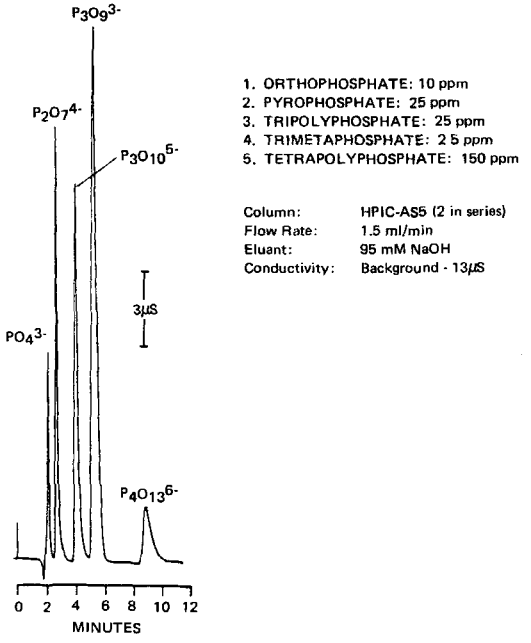

Fig. 13. Separation of polyphosphates (Ref. 11).

Anion Separation

The development of Ion Chromatography was a definite breakthrough where the chromatographic separation and quantitation of anions are concerned. Before the advent of Ion Chromatography the determination of simple anions involved laborious wet-chemical methods such as titrimetric, gravimetric,, or spectrophotometric, techniques. Thanks to the development of rigid, low-capacity anion exchange beads, efficient columns could be devised for this purpose. Fig. 12 is an example of the chromatographic separation of 9 common anions, which is completed within 6 minutes using standard chromatographic equipment with sensitive conductometric detection.

Anion separation has found many applications in the fields of environmental, agro, geo, and biochemistry, in hydro-metallurgy, etc. (20). Fig. 13 is just one example of the many uses, demonstrating that Ion Chromatography is advancing towards the speciation of complicated mixtures, such as that of the polyphosphates shown.

The subject of anion HPLC has recently been reviewed by Haddad and Heckenberg (21). They detail the various modes in which anion chromatography can be performed, and they list 34 different anions that can now be determined by this method.

Conclusion

This survey was not meant to be an exhaustive treatment of the entire field of Ion-Exchange Chromatography, which is amply documented in the literature and in a number of review articles. The present paper should mainly be taken as an overview of the field in general in order to show how the development of ion-exchange resins and the improved understanding of their mechanism were adapted to the frame of HPLC as well as the great variety of organic and inorganic compounds that can be separated and determined with this method.

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